HRI's Mission:

To direct, fund, promote and communicate horticultural research, which increases the quality and value of ornamental plants, improves the productivity and profitability of the nursery and landscape industry, and protects and enhances the environment.

cuttings should be maintenance of high rooting medium temperatures. Although the optimum medium temperature for adventitious rooting often cannot be rigidly defined (5), results reported herein for firebush indicate that maintaining the medium temperature between 29–39°C (85–101°F) yields excellent rooting.

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Anomalous Root Structure on Woody Plants *in vitro*[1]

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Abstract

The anatomical structure of *in vitro*-generated roots contrasted sharply with *ex vitro* roots produced on parallel treatments of micropropagated woody plant clones. *In vitro* roots contained enlarged, irregular, frequently pigmented cortical cells with numerous intercellular spaces, and only primary vascular system 4–5 weeks after initiation, while *ex vitro* roots of the same age were comprised of smaller, uniformly arranged cell layers and were developmentally further advanced (exhibited secondary vascular system development). Light exposure was not a major influence on anomalous structure of *in vitro* roots, other than pigmented cell frequency. *Ex vitro* rooting hormone stimulated production of thicker, more frequent root initials, although root structure beyond the point of emergence from the stem quickly transformed to the slender, compact character typical of *ex vitro* production.

Index words: auxin, Indole-3-butyric acid (IBA), Naphthalene acetic acid (NAA), photosynthetic photon flux (PPF), rhizogenesis, tissue culture

Species used in this study: Red Sunset Red maple (*Acer rubrum* L. 'Red Sunset'); River birch (*Betula nigra* L.); Sunrise border forsythia (*Forsythia* × *intermedia* Zab. 'Sunrise'), McIntosh apple (*Malus* × *domestica* Borkh 'McIntosh'); Red Ace miniature rose (*Rosa chinensis minima* 'Red Ace')

Significance to the Nursery Industry

In vitro rooting is sometimes preferred by tissue culture producers for two reasons: 1) it maximizes grower control over rooting conditions; and, 2) it simplifies shipping and transport of micropropagated plants to the nursery, since unrooted microcuttings are extremely tender. Alternatively, many large micropropagation labs have found that *ex vitro* rhizogenesis confers increased production efficiency, and lower costs. Often in the nursery industry, a grower may purchase micropropagated liners or young plants without knowledge of the method used during rhizogenesis of the microcutting at an earlier stage of production. Because the agar medium rooting environment is quite different than the transplant environment, the quality of the initial adventitious roots and framework root system on a woody plant may hinge on the method selected to induce rooting. Nursery growers should realize that acclimatization timing and subsequent handling procedures may be conditioned by the rooting method.

Introduction

The ability to propagate a superior woody plant clonal selection, by tissue culture or any other method, is a critical advantage in the nursery industry. The value of a cutting propagation method, however, hinges on the ability to successfully root and adapt new plantlets to field conditions. The decision to root woody microcuttings *in vitro* (while still in agar medium) or, alternatively, *ex vitro* (in high humidity and in soilless mix) can lead to significant differ-

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ences in root system morphology, root length, persistence, root number, and individual root diameter (7). These discrepancies have consequences not only for the acclimatization of the cutting, but also the later quality of the landscape plant.

In this report, we describe unusual anatomical features that characterize *in vitro* produced roots on five woody plants, and discuss their relevance in terms of the acclimatization process. In addition, we investigated the specific influences of light exposure *in vitro* and exogenous auxin *ex vitro* on adventitious root system initial development.

Materials and Methods

Shoot cultures of Red Sunset red maple (*Acer rubrum* L. 'Red Sunset'), River birch (*Betula nigra* L.), Sunrise border forsythia (*Forsythia* × *intermedia* Zab. 'Sunrise'), McIntosh apple (*Malus* × *domestica* Borkh 'McIntosh'), and Red Ace miniature rose (*Rosa chinensis minima* 'Red Ace') were the source of microcuttings for all rooting experiments. Proliferation media varied according to the plant species, and all cultures were grown in GA7 vessels (Magenta Corp., Chicago, IL) for 4–5 weeks under a PPF of 40–50 μmol m^{-2} s^{-1}, 24 hr photoperiod, at 25 ± 2°C (77°F) prior to use in experiments (6).

In vitro/ex vitro rooting methods. For *in vitro* root initiation, the basal 1.0–1.5 cm (0.4–0.6 in) of uniform microcuttings were inserted into 25 ml (0.85 oz) of agar-solidified medium containing no hormones (*Acer, Betula,* and *Forsythia*), 1.5 mg l^{-1} (.04 oz 1.06 qt^{-1}) IBA (*Malus*), or 0.1 mg l^{-1} (.003 oz 1.06 qt^{-1}) NAA (*Rosa*) in 25 × 150 mm glass culture tubes capped with a polypropylene closure (Magenta Corporation, Chicago, IL). Identical *ex vitro* counterparts were inserted into sterile moist silica sand; *Rosa* and *Malus* cuttings were first pretreated with 0.1% IBA rooting powder. *Ex vitro* treatments were contained in sealed high humidity clear plastic boxes; the seals were broken after 2 weeks to permit gradual acclimatization. Both *in vitro* and *ex vitro* rooting treatments were held in the same growth chamber at 25/21°C (77/70°F, at a PPF of 40 μmol m^{-2} s^{-1}, for 4–5 weeks. In separate experiments, *in vitro* rooting treatments were retained in the original rooting medium for up to 10 weeks prior to examination. At the end of the rooting period, *in vitro* plants were image analyzed by staging the entire test tube in front of a light box and viewing with a Sony CCD video camera (8). *Ex vitro* rooted plants were viewed in a similar manner after gently rinsing adhering sand from the root system. Image data was processed by an Imaging Technologies digitizer board housed in an IBM AT microcomputer, and Image Pro software (Media Cybernetics) to quantify overall root length and root system area (8, 9). Roots were then sectioned approx. 1 cm (0.4 in) from the root origin (the basal microcutting stem) using a vibrating microtone (Oxford vibratome) to examine anatomical composition. Fresh sections were viewed using an inverted Nikon Diaphot microscope at 10× with an attached video camera and imaging system as described above. Total and vascular cross-sectional areas were calculated, and the presence of unique cell types was recorded. Measurements were collected from 15 replicate microcuttings/species/treatment.

Influence of light during rooting. In separate experiments, microcuttings (10/species/treatment) of *Acer, Forsythia, Malus*, and *Rosa* were rooted *in vitro* (with the root zone in agar medium exposed to the ambient light in the culture room), or alternatively the basal portion of culture tubes was shrouded in aluminum foil wrap. *Ex vitro* treatments were prepared concurrently. Evaluations were conducted after 4–5 weeks as described above, and the experiments were repeated once.

Effect of in vitro *rooting hormone.* Microcuttings of *Rosa* and *Acer* were rooted *ex vitro* after pretreatment with 0.1% IBA rooting powder, or inserted into sand directly without auxin pretreatment. After 4–5 weeks, root systems and individual roots from 10 samples/treatment were examined as described above.

Results and Discussion

Since lateral roots did not occur *in vitro, ex vitro* root system area was significantly higher than *in vitro* for maple, apple, and rose 4–5 weeks after the start of rooting treatments. *In vitro* plants maintained vigorous shoot growth during the rooting process, whereas shoot growth was temporarily suspended while plants initiated roots *ex vitro*. Individual *in vitro* roots, however, were thicker with higher weighted density (an image measurement equivalent to fresh weight; 8) than their *ex vitro* counterparts. Examination of fresh adventitious root sections revealed that total root diameter for *in vitro* sections exceeded *ex vitro* root diameter by approx. 11% (rose) up to 37% (maple). The greater diameter of the fleshy *in vitro* produced roots was primarily a consequence of the looser arrangement of cortical parenchyma, with larger, hypertrophied individual cells and numerous intercellular spaces (Figure 1; Table 1). *In vitro* cortical, phloem, and parenchyma cells frequently had plastids containing chlorophyll and other pigments, and an abundance of starch grains. *Ex vitro* roots in contrast were non-pigmented, and exhibited uniform, compact cortical cell arrangement. Contrary to some previous reports (1, 2, 10), *in vitro* roots did have numerous, thickened root hairs, which were uniformly short as compared to the *ex vitro* roots with slender root hairs of irregular lengths (Table 1).

The most striking difference between *ex vitro*- and *in vitro*-produced roots was the lack of secondary vascular growth in the latter (Fig. 1). *Ex vitro* roots usually showed evidence of vascular cambium activity (and secondary xylem formation) in cross section, and were consistently arranged in either diarch, triarch, or pentarch stele patterns depending on the species (Table 1). Immature vascular systems revealed in parallel *in vitro* root cross sections were usually still in multiple scattered xylem bundles with variable stele patterns, and no secondary growth. Secondary tissue development *in vitro* was not just temporarily delayed as compared to *ex vitro* rooting treatments: root sections from microcuttings retained up to 10 weeks in the *in vitro* rooting media still failed to exhibit any advanced vascular development for any of the species examined. Vascular cambium activity was not initiated in these *in vitro* rooting treatments until transplant of the rooted microcuttings to *ex vitro* conditions.

In vitro roots sometimes were subtended by callus or emerged from callus for maple, birch, and apple microcuttings. Vascular connections between the new roots and the microcutting stem in these cases were often discontinuous. Adjacent *in vitro* adventitious roots would frequently be

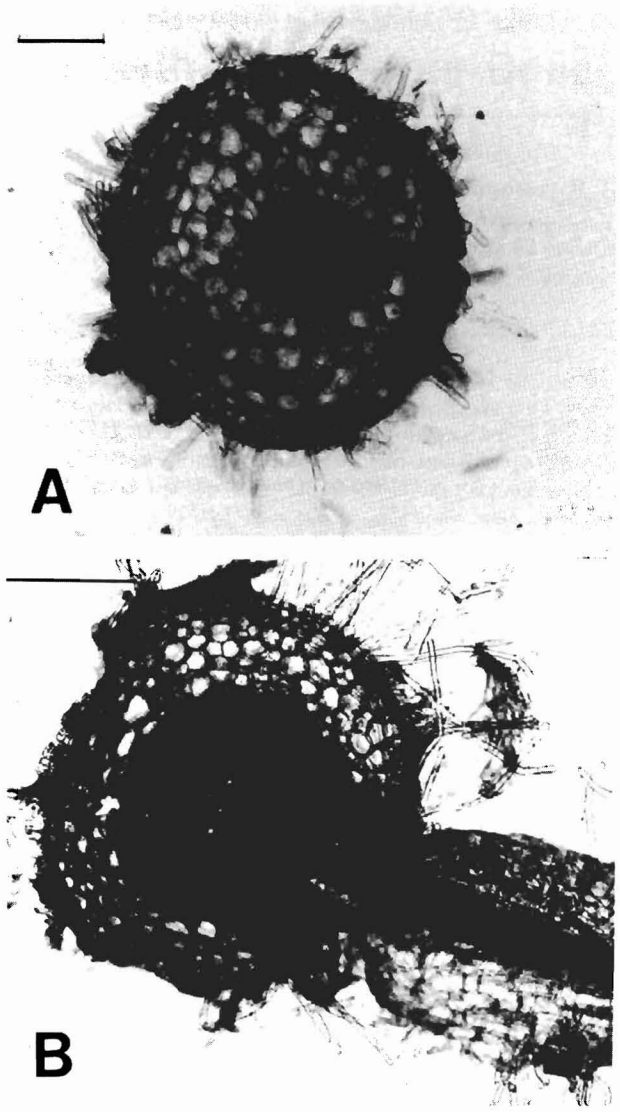

Fig. 1. Cross-section comparison of *in vitro* (A) and *ex vitro* (B) roots produced on maple microcuttings 5 weeks after the start of rooting treatments. *In vitro* roots (A) frequently contain plastids and starch grains in the cortical parenchyma, and thickened, short root hairs. *Ex vitro* roots (B) exhibited secondary vascular system development, and lateral root formation. Bar = 0.28 mm.

fused for up to 0.5 cm (0.2 in) from the point of origin. Neither callus nor root fusion was ever observed in any of the *ex vitro* rooting treatments. Some of the roots on *in vitro* microcuttings of birch and rose became blackened shortly after emergence, and failed to continue to elongate beyond 1–2 cm (0.4–0.8 in) while *in vitro*. Inspection of cross sections showed that the black color was restricted to a cuticular coating around the epidermal cells of the root. The *in vitro* root anatomy for these blackened roots was slightly more compact and uniform than for most typical (white) *in vitro* roots, although vascular development was still deficient.

The more compact, uniform anatomical structure of the *in vitro* roots that developed a blackened sheath (as compared to most *in vitro* roots) suggested that light may be a factor in the development of anomalous *in vitro* root anatomy. When light was partially excluded from the *in vitro* rooting zone with a foil wrap in our tests, the resultant root anatomy was slightly more uniform (slightly smaller and more compactly arranged cells) than light-grown roots, but the vascular system development remained retarded as compared to *ex vitro* roots (Table 2). The aluminum foil shroud, although it did not exclude light from the top surface of the medium, was used instead of charcoal to reduce light to the root zone, to avoid any interactions between charcoal and the medium components. Chlorophyll and other pigments, and large amyloplasts were observed only in the roots fully-exposed to light during initiation. There was no difference in rooting percentage between illuminated and shrouded *in vitro* treatments. Black-coated roots, which occurred occasionally in the light-exposed media, were not produced in any of the treatments with foil-wrapped bases. Activated charcoal and graphite suspended in the *in vitro* rooting medium have promoted rooting in various species. Since graphite does not absorb toxic substances like charcoal, its beneficial influence is assumed to be solely due to decreasing irradiance to the rooting zone (4). Khosh-Khui and Sink (5) reported that shading culture bases with black tape improved rooting of rose shoot tip cultures. These enhancements were reported in terms of overall rooting percentage, rather than root quality.

Pretreatment of microcutting stem bases with auxin prior to insertion into the *ex vitro* medium resulted in production of root initials of broader overall diameter (similar to *in vitro*-produced roots) at the point of emergence from the stem. Cortical cells within this initial segment of the root were large and slightly hypertrophic. However, within 0.75–1.0 cm (0.3–0.4 in) (*Acer*) and 0.5–0.75 cm (0.2–0.3 in) (*Rosa*), the adventitious roots reverted to the narrower, more compact anatomy characteristic of *ex vitro* root production. The development of vascular cambium, secondary vascular tissues, and advanced structural development (multiseriate epidermis and endodermis) were evident early in the development of the root.

The atypical *in vitro* root structure has a strong bearing on the microcutting acclimatization process. While *ex vitro*-produced roots adapt more readily to transplanting, *in vitro* roots are forced to undergo partial transformation in anatomical character in order to survive in soil (3, 7). In part, the transition period requires reversion of abnormal anatomical features (pigmented cells, hypertrophied cells, discontinuous vascular strands, etc.) to more typical root structure before secondary growth can occur. Under some conditions, adventitious root production *in vitro* does not result in any apparent production disadvantage during acclimatization, whereas in other cases the *in vitro* root system demands a longer acclimatization period and may be less tolerant of adverse conditions (7). In our experience, although *ex vitro* root systems have a greater area immediately following the rhizogenesis stage (due to lateral root production), the root systems of plants rooted *in vitro* eventually approach and even exceed the overall extent and area of *ex vitro*-rooted plants, during later stages of production. This may be in part be due to the increased shoot growth on *in vitro*-rooted plants in the greenhouse, which encourages more root development, or may be a consequence of residual hormone influence. Overall, however, the root to shoot ratio on the

Table 1. Common anatomical distinctions between *in vitro* and *ex vitro* produced roots of five woody plants, 4–5 weeks from the onset of the rooting phase.

Characteristic	*in vitro*	*ex vitro*
Intercellular spaces	many, large irregular	few, small
Cortical cell contents	many chloroplasts and other pigment containing plastids; abundant starch grains	no pigment; few starch grains
Endodermis	uniseriate	multiseriate
Vascular cambium, secondary xylem and phloem	none to very little	usually present
Stele	variable polyarch patterns	diarch, triarch, and pentarch most common, uniform pattern within a species
Cortical cell type	hypertrophic parenchyma cells, sometimes lysogenous	no cell hypertrophy evident; parenchymous and collenchymous cells
Epidermis	uniseriate only	uniseriate to multiseriate
Root hairs	short, thickened, uniform length, many fused together, usually abundant	generally much longer more variable in length slender, sparse
Callus	frequently accompanied root formation	not produced
Root system	brittle, thickened roots, some fused	slender, fibrous roots

Table 2. Effect of light exposure *in vitro* on adventitious root development for 4 woody plants, 4–5 weeks from the onset of the rooting phase.

Species	Treatment	Root system area (mm^2)	Root cross-sectional area (mm^2)	V/T[Z]
Acer[X]	In vitro-light	132.7 b[Y]	0.97 a	0.082
	In vitro-dark	143.0 b	1.01 a	0.095
	Ex vitro	182.3 a	0.38 b	0.230
Forsythia	In vitro-light	84.6 a	0.55 a	0.053
	In vitro-dark	65.4 a	0.47 a	0.067
	Ex vitro	60.2 a	0.44 a	0.148
Malus	In vitro-light	64.5 b	0.75 a	0.084
	In vitro-dark	53.5 b	0.71 a	0.068
	Ex vitro	80.2 a	0.58 b	0.122
Rosa	In vitro-light	60.0 b	0.34 a	0.098
	In vitro-dark	65.0 b	0.33 a	0.085
	Ex vitro	99.8 a	0.28 a	0.142

[Z]V/T = ratio of vascular system cross sectional area to total root cross sectional area.

[Y]Values are the mean of 20 replicate measurements per treatment. Significantly different means within each column are followed by a different letter, and were separated by LSD, 5% level.

[X]Each plant species was analyzed separately.

plants originally rooted in culture remains abnormally low in later production stages. *In vitro* rooting apparently presents less liability for herbaceous microcuttings (chrysanthemums, tomatoes, other herbaceous plants), probably since the *in vitro* rhizogenesis period is significantly shorter, and secondary vascular tissue is not typically produced.

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